INHIBITION OF FUNGI BY A SOIL STREPTOMYCETE

THE actinomycetes are abundant particularly in slightly alkaline soils rich in organic matter[1]. Thirty soil samples were collected from different localities in and around Calcutta and plated in Thornton[2] and Norris[3] media. Two hundred and fifty actinomycetes were isolated and tested for antibiotic producing capacities preliminarily by the agar cross streak and finally by the agar cup methods[4] of assay. Sixty-seven actinomycetes having antimicrobial activities were obtained. Of these one showed wide antifungal activity against a number of fungi including the phytopathogens and dermatophytes. Tentatively the organism was designated as *Streptomyces* sp. T_4.

The organism was grown on glucose-asparagine-peptone agar[5] slants at 28° C for 7 days and spores were collected in 10 ml sterile distilled water. A suspension of 24×10^6 spores/ml was used to inoculate 25 ml fermentation medium in flaks. The medium[6] contained sucrose 20·0, $NaNO_3$ 3·0, KCl 0·32, NaH_2PO_4 0·032 and $MgSO_4, 7H_2O$ 0·016 g/l (pH 7·5). Incubation at 28° C for 7 days resulted in optimum production of the antibiotic. The inhibitory activity of the antibiotic is presented in Table I.

TABLE I

Antifungal activity of the antibiotic produced by Streptomyces *sp. T_4 as determined by the agar cup method of assay*

Test organism	Minimum inhibitory concentration (μg/ml)
Candida albicans	32·0
Saccharomyces cerevisiae	19·0
Sporotrichum schenkii	100·0
Cryptococcus neoformans	40·0
Microsporum canis	100·0
M. gypseum	50·0
Epidermophyton floccosum	115·0
Nocardia asteroides	175·0
Trichophyton tonsurans	130·0
T. rubrum	125·0
T. violaceum	140·0
Aspergillus niger	45·0
Curvularia lunata	19·0
Helminthosporium sativum	12·5
H. oryzae	16·0
Alternaria solani	21·0
Penicillium chrysogenum	16·0
Syncephalastrum sp.	16·0
Trichoderma viridae	50·0

The active principle was extracted from the culture broth with *n*-butanol. The extract was dried, dissolved in methanol and repeatedly precipitated with acetone. Finally the precipitate was dried *in vacuo* and the active principle was obtained as light yellow powder. The antibiotic is soluble in water, methanol, pyridine and glacial acetic acid. It melted at 150–152° C. It is homogeneous as evidenced by unidimensional descending paper chromatography. Rf values obtained with different solvent systems in bioassays against *Saccharomyces cerevisiae* were—*n*-butanol-acetic acid—water (4 : 1 : 1) 0·68; *n*-butanol—ethanol—water (1 : 1 : 1) 0·87; ethanol—water (7 : 3) 0 85; *n*-butanol saturated water 0·025 and water saturated *n*-butanol 0·25. The antibiotic is a non-polyene with a peak at 210 nm in the UV absorption spectrum[7].

The ability of *Streptomyces* sp. T_4 in inhibiting the dermatophytes, spp. of *Sporotrichum*, *Epidermophyton*, *Cryptococcus*, *Microsporum* and *Trichophyton* appeared to be interesting. Attempts are being made to purify and characterize the active material further so as to compare its potency with griseofulvin and other non-polyenes.

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INDUCTION SYNCHRONY AND NUCLEAR BEHAVIOUR IN *FUSARIUM OXYSPORUM*

THE germination of fungal spores in general has been reviewed by Cochrane[1] and Hawker[4] while studies of Marchant and White (1965), Gracia *et al.*[3] and Punithalingam[6] throw light on dynamics of conidial swelling, germ tube initiation, fine structure and cytology of *Fusarium culmorum*. It is well known that synchronous cultures are superior to any single cell technique, for they open the cell cycle to a wide range. Synchrony was induced in the germinating conidia

of *Fusarium oxysporum* f. sp. *melonis* (M 15D) a highly pathogenic strain isolated from melons by using a known precursor- deoxyadenosine.

Single cell cultures were maintained on modified Asparagine medium. Conidial suspension was prepared from 10–15 days old cultures and Erlenmeyer flasks (250 ml) containing 100 ml of modified Asparagine medium were inoculated. The medium (10 ml) was pipetted out from these flasks at each time into an equal volume of trichloroacetic acid kept at 0° C for half an hour. The technique of Rosenberger and Kessel[7] was employed to stain the conidia, where under a UV microscope, the nuclei appeared green to light green and the germ tubes red. Deoxyadenosine (2 mM) was introduced during the second nuclear division. In *Fusarium*, the macroconidia are 3–5 celled, fusoid wherein each cell has a nucleus while the microconidia are uninucleate and 1-celled. The chlamydospore (sedentary) is thick walled, single celled, oval to spherical and uninucleate.

Germination is best seen in slide cultures and it starts after an hour. Septa and vacuoles become conspicuous with the increase in permeability (Fletcher[2]). Germination is not synchronous. The germ tubes develop septa with varying interseptal region (6 h). The first nuclear division in either conidia takes place in 3–4 h and the binucleate condition remains for 3–5 h which in a natural sequence divides asynchronously to have 3 nuclei. Thus the 2nd and 3rd divisions during germination is not synchronous. Treatment with deoxyadenosine after the first division (4 h and releasing after 6 h) results in a partial synchrony (Mitchison and Creanor[3]). Deoxyadenosine acts as a block and accumulates by synchronous division. The uninucleate chlamydospores divide synchronously up to 16 nuclei. The behaviour and division of neclei in (i) macroconidia and (ii) microconidia are different. Further, chlamydospores behave totally unlike the conidia. This behaviour is highly suggestive of the existence of more than one nuclear cycle. The synchronous division of the chalmydospores appears to be biologically significant from the point of pathogenicity (inoculum). For, *Fusarium culmorum*, Graciá *et al.*[3] reported the absence of the nuclei or any other structure that might be considered as chromosomal in nature in the end cells of the conidia (macro-) while studies of Punithalingam[6] revealed that the end cells of the conidia contained nuclei as found in this strain (*cf.* Table I). The asynchronously dividing nuclei of this strain, can be induced to divide synchronously, indicates that the events in the cycle are closely associated with DNA synthesis and division.

TABLE I

The effect of deoxyadenosine on the germinating conidia of Fusarium oxysporum

	Controlled						Treated					
	Macroconidia			Microconidia			Microconidia			Chlamydospores		
Hrs.	nuclei	germ-tube	% germination	nuclei	germ-tube	% germination	nuclei	germ-tube	% germination	nuclei	germ-tube	% germination
0	(1, 1, 1, 1)	0	0	1	0	0	1	0	0	1	0	0
1	(1, 1, 1, 1)	0	00	1	0	0	1	0	0	1	0	0
2	(1, 1, 1, 1)	2·5	1–2	1	0	0	1	0	0	1	0	0
3	(1, 1, 1, 1)	8–35	2–5	1	0	0	1	2–7	1-2	2	0	0
4	[(1 + 1)/2, 1 + 1]	30–100	15	2	5–10	8	2	5-15	6-10	2	0	0
5	[(1 + 1)/2,...]	100–225	25	2	10 -25	10–15	2	5·35	15	2	0	0
6	[(1 + 1)/2,...]	200 -325	38	3	15·50	25	2	15·55	25	4	5 10	10
7	[(1 + 1 + 1)/3, ...]	325–500	55	3	45·100	35	4	50 150	35	4	5 15	15
8	[(1 + 1 + 1)/3, ...]	500-1 mm	75-80	5	65·160	47	4	65 170	50	8	10 25	20
9	—	..	..	5	150 250	65	8	150 300	80	8	20 30	20
10	—	..	mycelia	5	300	78	..	..	..	8	upto 45	25
11	—	..	..	7	300	80	..	..	16	16	65	25

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BACTERIAL LEAF SPOT OF BETELVINE (*PIPER BETLE* L.) INCITED BY *XANTHOMONAS BETLICOLA* PATEL *ET AL.* IN KERALA

A BACTERIAL disease of endemic pattern was reported on betelvine in Kerala by the farmers during the three years (1975–77). This disease was very severe in the affected gardens involving the whole plantation. Preliminary studies suggested that the organism can go to Peppervine (*Piper nigrum* L). In view of the potential crop losses, studies were taken up on the symptomatology of the disease and identity of the pathogen. The results are presented in this note.

A bacterial leaf spot on betelvine was reported by Patel *et al.*[1] for the first time in India as early as 1951 and named the pathogen as *Xanthomonas betlicola*. This was considered as a minor disease, even though there were subsequent reports of its occurrence in 1971 from Jabalpur area of M.P.[2].

Symptomatology of the Disease

In the present study a pure culture of the organism was established and the pathogenicity proved. Both under natural and artificial inoculated conditions, the symptoms of the disease were studied. The infection begins as minute water soaked spots all over the leaf blade delimited by veins. Several of them coalesce to form larger irregular brownish spots. The advanced lesions are invariably accompanied by yellowish halos and result in ultimate defoliation. The symptoms are found to vary with varieties.

In certain cases the infection is confined to the leaf margins, diffused and quickly spreading in an irregular fashion (Figs. 1, 2 and 3). Often greyish black lesions are seen on the stem and petioles.

FIG. 1. A healthy and infected leaf in a diseased plantation. Note the vines without leaves.

FIG. 2. Symptoms produced by the pathogen on peppervine.

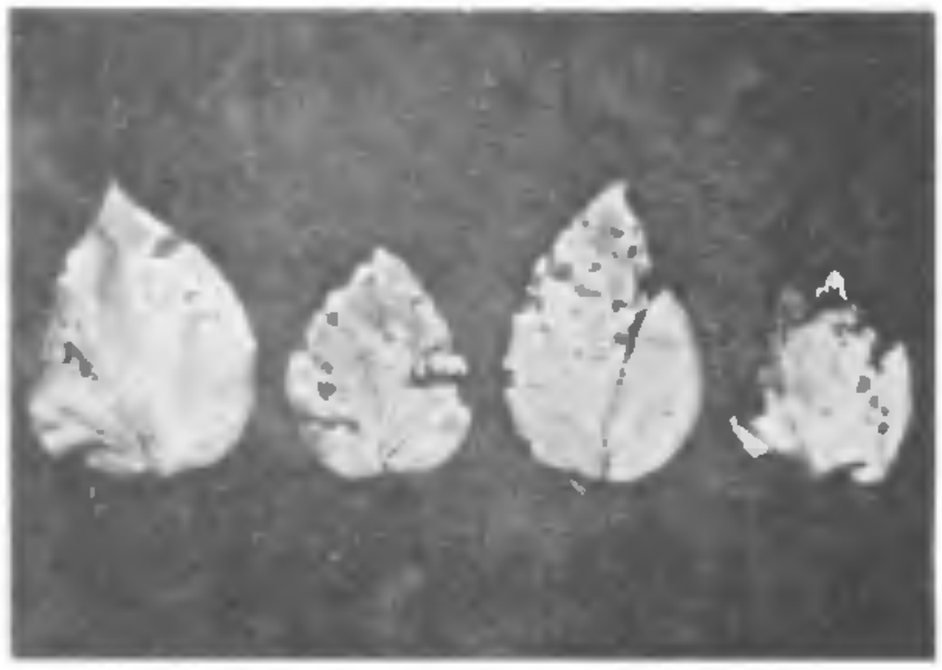

FIG. 3. Progressive stages of the disease on betelvine.

On artificially inoculated peppervine, the symptoms appear as minute necrotic spots with yellow halos.